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Hydroethidine™

Catalog Number 17084

- Enters and stains living cells without cellular trauma.
- Double staining system stains cytoplasm blue and chromatin red.
- Excellent cellular retention - remains incorporated in chromatin with virtually no leakage.
- Essentially non-toxic - shows no toxicity at level useful for visualizing chromatin.
- Chromatin fluoresces red when excited by visible and/or UV light - excellent for flow cytometry applications.
- May also be useful to study certain dehydrogenative metabolic activities.
- Useful in monitoring hybrid cell formation.

Technical Specifications:

Molecular Formula:	$C_{21}H_{21}N_3$
Molecular Weight:	315.5
Melting Point:	203°C - 206°C
Solubility:	Slightly soluble in physiological buffers
Handling precautions:	Frameshift mutagen which intercalates DNA and RNA
Storage:	Store cold in the dark, under nitrogen

Background:

Hydroethidine™ is an uncharged racemic fluorescent compound* produced by the reduction of ethidium bromide. It is readily taken up by living cells and shows blue fluorescence in the cytoplasm when viewed with UV (370 nm) light. Within the living cell, Hydroethidine is enzymatically dehydrogenated, in part, to form ethidium, which becomes locked in the cell by virtue of its cationic nature. The ethidium intercalates into the DNA and is red fluorescent when excited by either visible (535 nm) or UV (370 nm) light.

Staining Procedures:

The concentrations and times employed for the staining solutions have been determined for the fibroblastic type of cells. Other cell types may require higher or lower concentrations of the stain and different staining times. The optimum concentration and time should be determined for each cell type.

Stock solution: 7 mg of Hydroethidine is dissolved in 1 ml of N,N-dimethylacetamide.

Working solution: To 10 ml of phosphate buffer saline (PBS) (0.02M sodium phosphate, 0.15M NaCl, pH 7.4) add 20 µl of the stock Hydroethidine™ solution and mix immediately. Filter the solution if it is not perfectly clear.

Staining of Surface Adherent Cells:

The cells that attach to the surface of the cultured flask are grown on coverslips in petri dishes and are usually stained 24 hours or longer after seeding when they are firmly attached to the glass surface.

1. Remove the medium and wash the cells three times with the same buffer used for the preparation of the staining solution. Remove all the buffer.
2. Add the staining solution and leave for fifteen minutes at room temperature, protecting the cells from light with aluminum foil.
3. Remove the staining solution and wash the cells three times with the buffer.
4. Deposit one drop of the buffer on a slide. Remove the coverslip from the petri dish, wipe gently the side opposite to the cells with a tissue and place the coverslip vertically on the slide with the cells facing the drop of buffer. Drop the coverslip and gently remove excess liquid with a tissue.

Staining of Cells That Grow in Suspension:

1. Centrifuge the cell suspension for 5 minutes.
2. Remove supernatant and wash with the appropriate buffer. Remove all of the buffer.
3. Add the staining solution to the cell pellet . Gently suspend the cells in the staining solution and leave the stain at room temperature for 15 minutes, protecting the cells from light with aluminum foil.
4. Centrifuge the cell suspension and remove the staining solution. Wash the pellet once with buffer. Resuspend the cells in a small volume of the buffer.
5. Place a drop of the suspended cells on a slide and cover with a coverslip. Gently remove excess liquid with a tissue.

Alternatively, instead of observing the cells immediately, the stained cells can be placed in medium again and cultured for various periods of time to follow the destaining process and visualize those structures which hold to the dyes more firmly. For the destaining procedure it will be necessary to maintain sterile conditions. The staining solutions should be filtered through a 0.22 µm sterile Millipore filter unit.

Fluorescent Observation:

To observe the blue fluorescence of the cell cytoplasm and the reddish color of the nucleus, use a UV exciting light (370 nm) and a barrier filter passing light at 420 nm. See Photo A. The chromatin staining (red) is best observed with an excitation filter at 535 nm and a barrier filter at 585 nm. Under these conditions the cytoplasm is essentially non fluorescent. See photo B.

Other Fluorescent Labels

Polysciences offers a wide range of fluorescent probes and labels, many of which are listed in Data Sheet 250. Our Fluoroboras, described in Data Sheet 277 are also useful in staining living cells.

Storage and Handling Precautions:

Hydroethidine™ is a frameshift mutagen which intercalates with DNA and RNA. *Handle with care.* It may cause injuries and problems to eyes, skin, and respiratory tract, so avoid contact with eyes and skin and breathing of vapors. Wear protective goggles, gloves, and clothing. Use only with adequate ventilation. Avoid prolonged and repeated exposure. Wash thoroughly after handling. In case of accident, flush eyes or skin with plenty of water for at least 15 minutes. Call a physician immediately, especially if eyes are affected. *Store cold in the dark.*

Ordering Information:

Cat. #	Description	Size
17084	Hydroethidine™	50mg

To Order:
In The U.S. Call: 1-800-523-2575 • 215-343-6484
In The U.S. FAX: 1-800-343-3291 • 215-343-0214

In Germany Call: (49) 6221-765767
In Germany FAX: (49) 6221-764620

Reference:

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3. Saiki, I., Bucana, C.D., Tsao, J.Y., and Fidler, I.J.: Quantitative fluorescent microassay for identification of antiproliferative compounds. JNCI, **77**, 1235 (1986)
4. Bucana, C., Saiki, I., and Nayer, R.: Uptake and accumulation of vital dye hydroethidine in neoplastic cells. J. Histochem. Cytochem., **34**, 1109 (1986).
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*Patent pending ™ Prescott Laboratories